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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/501,777

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John Robert Birch

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EXAMINER

EPSS -SMITH, JANET L

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/501,777	Applicant(s) BIRCH ET AL.	
	Examiner Janet L. Epps-Smith	Art Unit 1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 20 July 2009.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 32-41 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 32-41 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 07/02/2009 has been entered.
2. Claims 32-41 are under examination.
3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Response to Arguments

Claim Rejections - 35 USC § 103

4. Applicant's arguments with respect to the rejection of claims 1, 3-7, 10, 14-15, 17-24, 27-29, and 32-35 under 35 U.S.C. 103(a) as being unpatentable over Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record) and Gawlitzek et al. (see IDS filed 5/07/08) and Hermitin et al., are moot in view of the new grounds of rejection set forth below.
5. Claims 32-41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of

record) as evidenced by Barsomian et al (U.S. Patent No, 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record), Schneider et al. (Journal of Biotechnology, Vol. 46, pages 161-185, 1996), Gawlitzek et al. (see IDS filed 5/07/08) and Hermentin et al. (US 6096555).

6. Applicant's arguments filed 07/02/2009 and 08/17/2009 have been fully considered but they are not persuasive. Applicants have devoted the majority of their argument on the assertion that Gawlitzek et al. did not confirm the findings of Schneider et al. and other references cited in the article regarding the effects of ammonia on the sialylation and/or glycosylation of N-glycan charge of glycosylated proteins, and therefore teaches away from the claimed invention.

7. However, contrary to Applicant's assertions, the disclosure of Gawlitzek et al. did not contradict the findings of these references nor did Gawlitzek et al. dismiss the previous findings, instead Gawlitzek et al. offers up several rationales why they were not able to precisely confirm the prior observations. See for example, page 645, last few paragraphs, wherein it states that "[D]ifferences in the cell line used (CHO vs. BHK), the glycoprotein produced (e.g., protein-specific glycosylation, accessibility of glycosylation site), and the cell culture process (batch vs. perfusion culture) might be responsible for the different results." Therefore, Applicant's assertion that the Gawlitzek et al. reference teaches away from the claimed invention is inaccurate.

Applicant's arguments are not supported by evidence. Absent evidence to the contrary, the ordinary skilled artisan at the time of the instant invention reading the teachings of Schneider et al. and Gawlitzek et al. would have had a reasonable

expectation of success in designing a method wherein controlling the levels of glutamine in a cell would have provided a means of reducing the excessive formation of ammonia, and therefore would have provided a means for controlling the detrimental effects of excessive ammonia on cells as described in Schneider et al. See Table 1 of Schneider et al. which clearly suggests that excessive ammonia in cells leads to a decrease in sialylation and glycosylation, and furthermore page 176 of Schneider et al. provides express motivation for controlling glutamine availability as a means for regulating the levels of ammonia in cells.

8. As stated in the prior Office Actions:

Wilson et al. teach the use of a recombinant DNA sequence, using glutamine synthetase as a selectable marker in glutamine auxotrophic cells to express tissue plasminogen activator (tPA; see page 8, lines 4-33; pages 9-11; Figure 3) Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

Bebbington et al teach mouse and rat lymphoid cell lines that can be transformed to glutamine independence by incorporating a gene encoding glutamine synthetase (GS) so that the cells can grow in glutamine-free medium. Bebbington et al teach that the cell preferably contains a gene coding for a heterologous protein in which the gene is encoded on a separate vector (see column 2, lines 8-20, 25-30 and 44-51, for example), which meets the limitation of exogenous DNA sequences being located on more than one DNA construct. Bebbington et al teach examples of heterologous proteins as human growth hormone, or tPA or tissue inhibitor of metalloproteinase (see column 2, lines 31-36, for example). Bebbington et al teach that the expression of the heterologous protein is substantially increased by selection for GS gene amplification (see column 2, lines 52-55, for example), which meets the limitation of GS as a selection marker. As stated above, Barsomian et al disclose that tPA is a sialylated glycoprotein (see column 2, lines 49-52, for example).

Bebbington et al do not teach a human glutamine-auxotrophic cell. Bebbington et al do not teach an exogenous sequence encoding a sialylated protein that further comprises a selectable marker such as claimed. Bebbington et al do not teach growing the cells in serum-free medium.

Brandt et al teach human cells (HT1080) for the preparation of human proteins in order

to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation (see column 1, lines 12--17, for example) which meets the limitation of glutamine-auxotrophic human cell line or an immortalized fibrosarcoma HT1080 cell line. Brandt et al teach the advantage of using serum free culture medium for culture of human cells because purification of proteins from serum free culture is substantially easier and has no danger of contamination with animal pathogens (see column 2, lines 22-34, for example) which meets the limitation of a human cell growing in a serum free culture. Brandt et al teach that it is advantageous to use a human cell-line that synthesizes a desired protein with a glycosylation protein, especially a sialic acid protein comparable to that of the naturally occurring target protein (see column 3, lines (19030, for example).

Brandt et al also teach that a negative or positive selection marker or amplification gene can be included and can be DHFR, adenosine deaminase, ornithine decarboxylase or a thymidine kinase gene (see column 4, lines 56-65, for example)," which meets the limitation of a gene further comprising a selectable marker selected from the group consisting of DHFR, adenosine deaminase, asparagine synthetase, aspartate transcarbamylase, metallothionein-1, ornithine decarboxylase, P-glycoprotein, ribonucleotide reductase, thymidine kinase and xanthine-guanine phosphoribosyl transferase (claim 35).

Brandt et al teach an HT1080 cell line (see column 8, lines 24-50, for example), which meets the limitation of a glutamine-auxotrophic human cell line or an immortalized fibrosarcoma HT1080 cell line (claim 37).

Brandt et al teach that the cells are cultured in serum-free medium and in suspension (see column 6, lines 25-42, for example). Bebbington et al teach that cells can be transformed to glutamine independence by incorporating a gene encoding GS so that the cells can grow in glutamine-free medium. Therefore, the cells made obvious by Bebbington et al in view of Brandt et al would be capable of growing in suspension in serum-free and glutamine-free medium (claims 32-36).

Brandt et al teach that the cells were cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see column 6, lines 44-63, for example), which meets the limitation of a process for the production of a sialylated protein in a glutamine-auxotrophic human cell in a serum free medium and recovery of the protein.

As discussed above, Bebbington et al in view of Brandt et al render obvious a cell capable of growing in serum free and glutamine free medium and would also render obvious a process for producing a sialylated protein by culturing a glutamine-auxotrophic cells wherein the culture medium is serum-free and glutamine free (claims 10-11). Brandt et al teach production of a protein such as EPO using an HT1080 cell line (see column 8, lines 24-50, for example), which meets the limitation of a process

wherein the cells is a glutamine-auxotrophic human cell line or an immortalized fibrosarcoma HT1080 cell line (claims 37-41).

None of the cited references teach a method for increasing sialylation and/or N-glycan charge of a glycosylated protein. Furthermore, none of the cited references teach that sialylation is defined by N-glycan charge or that the sialylated protein comprises tri-, tetra- or pentasialo glycoforms of N-glycan.

Schneider et al. teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine. Schneider et al. further teaches that increased amounts of ammonium in cells lead to a decrease in terminal galactosylation and sialylation of recombinant GCSF (see Table 1, page 163). Schneider et al. further teach that it is a widely held view that the most important inhibitory substance accumulating in cell cultures is ammonia, and that a reduction in ammonia levels has to be overcome in order to achieve the successful economic scale-up of such processes for the production of high value proteins (page 165). According to this reference the main source of accumulating ammonia in mammalian cell cultures is amino acid metabolism, particularly that of glutamine. Glutamine has been shown to be the most major nutrient, in addition to glucose, for most growing animal cells in culture. Glutamine therefore serves as a protein constituent and as the main energy source (see page 165, 2nd col). At page 176, Schneider et al. teach that one means of controlling excessive ammonia formation is to control the addition of glutamine in cells by limiting glutamine availability.

Thus, the reference sets forth a correlation between increased ammonia accumulation and decreased sialylation and/or glycosylation of a protein, and further suggests that limiting the availability of glutamine in a cell would result in decreased ammonia accumulation. Page 176, 2nd ¶ of Schneider et al. teach the following:

"Bols et al. (1995) reported the successful growth of several fish cell lines in glutamine-free media. Fish cells, in contrast to mammalian cells, do not require glutamine. Thus they could be grown in media deficient of glutamine, glutamate and α -ketoglutarate without any adaptation. The observed differences with mammalian cells must be due to differences in the biochemistry of fish cells."

Gawlitzeck et al. teach that in the culture of mammalian cells, the metabolite ammonium is produced as a by-product of glutamine metabolism and the thermal degradation of glutamine. Gawlitzeck et al. further teaches that increased amounts of ammonium in cells leads to a decrease in terminal galactosylation and sialylation of TNFR-IgG.

Hermentin et al teach a process for characterizing the glycosylation of glycoproteins based on a hypothetical charge number N. Hermentin et al teach that it is important to reliably determine the degree of glycosylation or sialylation in glycoproteins, such as erythropoietin, in order to gauge bioavailability/biological activity of a protein for therapeutic use. Hermentin et al discloses that when erythropoietin is incompletely glycosylated, it is quickly cleared from the blood circulation and would not be biologically useful (see column 1, lines 6-15, 28-45 and column 2, lines 14-25, for example). Hermentin et al teach that it is crucial to determine the distribution of glycan groups exhibiting differing degrees of sialylation to be able to index the bioavailability of a glycoprotein. Hermentin et al teach that the N charge of a glycoprotein is determined in part by determining the percentage of trisialo, tetrasialo and pentasialo ranges (see

column 3, lines 27-50, column 4, lines 27-35, column 5, lines 4-12, for example). Hermentin et al teach that the N-glycan charge value was determined for EPO and erythropoietin is comprised of trisialylated N-glycans and tetrasialylated glycans (see column 12, lines 43-52, for example), which meets the limitation of EPO as a sialylated protein comprising tri and tetrasialylated glycoforms defined by N-glycan charge and a process of defining sialylation by N-glycan charge.

It would have been obvious to the skilled artisan at the time the invention was made to combine the cited references in the design of the instant invention. One of ordinary skill in the art would have been motivated to modify the method taught by Bebbington et al from making a rodent glutamine auxotrophic cell to produce an exogenous sialylated protein such as tPA to a human cell as taught by Brandt et al to produce a human exogenous sialylated protein because Brandt et al disclose that human cells are preferred for the preparation of human proteins in order to produce human proteins in economical yields in a form that is suitable for production of a pharmaceutical preparation. The motivation to make a human glutamine auxotrophic cell that is transfected with a glutamine synthetase sequence and a separate vector comprising a sequence for an exogenous sialylated protein is the expected benefit of being able to use the cells to synthesize a target protein with a sialic acid moiety glycosylation pattern that is comparable to that of the naturally occurring target protein (see column 3, lines 23-30, for example). Moreover, Wilson et al. expressly teach the exogenous expression of glutamine synthetase in glutamine auxotrophic cells.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method and cell taught by Bebbington et al and incorporate a second exogenous DNA sequence as a selection marker and amplification gene because Brandt et al teaches clone selection and gene amplification using a positive or negative selection marker. The motivation to use a selection marker and amplification gene such as DHFR to make a cell is the expected benefit as disclosed by Brandt et al of being able to use a gene with a sensitivity for a selection agent in order to increase the expression of an gene to be produced by culturing the cell in the presence of increasing concentrations of a selection agent (i.e. methotrexate) (see column 10, lines 36-44, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell wherein these exogenous DNA sequences are located on more than one DNA vector construct, because similar methods have worked previously in the references cited. Claim 28 appears to recite limitations regarding the order of the vector transformations. Limitations of this type would amount to routine optimization of the method. Therefore, Bebbington et al in view of Brandt et al render obvious a method to produce the glutamine-auxotrophic human cell of claim 32.

It also would have been obvious to the skilled artisan at the time the invention was made to modify the method taught by Bebbington et al and make a glutamine auxotrophic cell that is capable of growing in serum-free medium because Brandt teach the importance of serum-free medium for cultivation. The motivation to use a serum-free medium is the expected benefit of being able to reduce the danger of contamination of the protein produced with animal pathogens that might be introduced by using animal

serum (see column 2, lines 22-35, for example). There is a reasonable expectation of success to make a human glutamine auxotrophic cell with these limitations, because a similar method worked previously in the references cited. Therefore Bebbington et al as evidenced by Barsomian et al in view of Brandt et al render obvious a method to produce the glutamine-auxotropic human cell of claim 32 wherein the cell is further adapted to growth in serum free medium as claimed in claim 36.

It would have been obvious to the skilled artisan at the time the invention was made to determine the N-glycan charge for the sialylated protein such as EPO or tPA being produced by the cell because Hermentin et al teach that it is important to know the degree of glycosylation of recombinant therapeutic proteins such as erythropoietin, since slightly altered glycosylation patterns can drastically effect the activity of the therapeutic protein. The motivation to determine the N-glycan charge is the expected benefit of being able to determine the degree of glycosylation in a simple, reliable /manner suitable for replacing the methods previously known in the art for determining the bio and ability of a therapeutic protein before use. There is reasonable expectation of success in combining the protein production method using a glutamine auxotrophic cell rendered obvious by Bebbington et al in view of Brandt et al with the methods taught by Hermentin et al and use a glutamine- auxotropic human cell transfected with an exogenous DNA sequence encoding a glycoprotein, such as erythropoietin, to produce and recover erythropoietin and determine its bioavailability via N-glycan charge, because these methods have worked before in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time

the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention. Therefore, Bebbington et al as evidenced by Barsomian in view of Brandt et al and further in view of Hermentin et al render obvious a cell and a process for the production of a sialylated protein comprising culturing a glutamine-auxotrophic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-, or tetrasialo glycoforms of the glycan defined by an N-glycan charge.

Additionally, it would have been obvious to the ordinary skilled artisan at the time of the instant invention, to modify the teachings of Wilson et al. (WO 87/04462 A1) or Bebbington et al (U.S. Patent No. 5,891,693, of record) as evidenced by Barsomian et al (U.S. Patent No. 5,238,821, of record) in view of Brandt et al (U.S. Patent No. 6,395,484, of record) by the teachings of Schneider et al. and Gawlitzek et al. The ordinary skilled artisan seeking to increase the sialylation and/or N-glycan charge of a glycosylated protein, would have been motivated to express the desired protein in a cell without adding glutamine, since the prior art clearly teaches that ammonium, a byproduct of glutamine metabolism has a direct effect on the level of sialylation and/or N-glycan charge of a glycosylated protein.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janet L. Epps-Smith whose telephone number is 571-272-0757. The examiner can normally be reached on M-F, 10:00 AM through 6:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Janet L. Epps-Smith/
Primary Examiner, Art Unit 1633